GENE ACTION IN PARAMECIUM[1]

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I. INTRODUCTION

Gene action in *Paramecium aurelia* has been investigated (Sonneborn, '43a) most fully in the case of the action of the gene involved in the determination of the difference between "killers" and "sensitives." One race of this species makes the fluid in which it lives poisonous to nearly all other races of *Paramecium*. This race is thus a "killer." Killers are invariably resistant to their own poison. Races affected by this poison may be called either "sensitives" or "non-killers," for sensitives are never killers. Likewise, non-killers are never resistant. The killer character is determined by the combined presence of a dominant gene, K, and a cytoplasmic factor, kappa. Killers always have kappa; without kappa, clones are always sensitive non-killers. Killer clones also always have, in addition to kappa, gene K; but non-killers, lacking kappa, may have either K or its recessive allele, k. Thus, neither K nor k can initiate the production of kappa. Nevertheless, there is some relation between these genes and kappa, for clones containing kappa always have K and are never homozygous for k. The role of the genes is shown by observations on homozygous recessives (kk) produced by the self-fertilization of heterozygous killers (Kk plus kappa). In these recessives kappa is retained for a few fissions, during which the cells remain killers; hence, cells are killers when kappa is in the cytoplasm and K is absent from the new nuclei (though still present in disintegrating parts of the old nucleus). However, after a few fissions, kappa disappears and the clone becomes and remains permanently non-killer. Hence, kappa is not independently self-multiplying; it depends upon gene K for its maintenance and increase. The role of the genes is further shown by introducing kappa into non-killers. If the non-killer has the genotype kk, the resulting clone is still a non-killer; but if the non-killer has the genotype KK or Kk, introduction of kappa results in its maintenance and increase, yielding a clone of killers. Hence in relation to gene K, kappa acts something like a primer in a pump: some kappa is put in and more comes out. K seems to be like a pump that will not work without being primed. The action of gene K in controlling increase of kappa is thus dependent upon a cytoplasmic primer, kappa itself.

The work just summarized suggests that an understanding of the action of gene K might be acquired by a detailed investigation of its relations to kappa. An investigation of these relations is reported in a paper now in press (Sonneborn,

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'45), in which a hypothesis of gene structure and action is induced from the results of the experimental analysis. The main features of this hypothesis, however, were suggested in the first place by the results summarized above and certain other facts known much earlier; the experimental analysis was in fact designed specifically to test the hypothesis. I shall therefore take this opportunity to point out how the hypothesis was indicated by the available information and how it was put to experimental test. This will make it possible to put on record pertinent material not presented in the other paper.

II. INFORMATION LEADING TO A HYPOTHESIS OF GENE STRUCTURE AND ACTION

The hypothesis was suggested in the first place by an attempt to account for the observed striking difference between the gross genetic phenomena in different varieties of *Paramecium aurelia*. In variety 4, all characters (killers, mating types, antigens) studied show essentially the same system of determination and inheritance. In every case, a cytoplasmic factor intervenes between gene and character. The gene alone cannot initiate production of this factor, though maintenance and increase of the factor are under the control of the gene. On the other hand, the characters (mating types, antigens) studied in variety 1 are determined by genes whose action does not depend upon cytoplasmic primers. Of the other varieties of *P. aurelia* thus far studied, some show the system characteristic of variety 1; the rest show the system characteristic of variety 4.

Two reasons make it appear unlikely that this difference between the genetic phenomena in the two groups of varieties could be due to any very profound difference in the genes and their mode of action. One reason is the extremely close relationship of the varieties; the other is the apparent identity in the two groups of varieties of the cytological processes on which the genetic phenomena must depend.

The seven varieties of *P. aurelia* thus far discovered differ little, if at all, morphologically. They are so much alike that they would never have been recognized without the discovery of mating types. They are, in fact, defined simply on the basis of sexual isolation. Within each variety, every individual belongs to one or the other of two mating types which interbreed freely; but neither mating type in one variety interbreeds with either mating type in any other variety. (Recently exceptions to this rule have been discovered, but they do not essentially alter the effectual isolation of the varieties.) Clearly, the diverse varieties are so closely related that they could hardly have diverged appreciably in anything so fundamental as gene structure and action.

Likewise, the visible cytological features of the fertilization processes are identical in the different varieties. In all varieties the diploid micronuclei in each conjugant undergo two meiotic divisions and all the resulting haploid nuclei disintegrate except one. This one goes through a third (equational) division, the two products of which are the gamete nuclei. One gamete nucleus in each con-

jugant passes into the mate and fuses with the gamete nucleus that has remained in the mate. This fertilization nucleus, in which the diploid condition has been restored, gives rise by ordinary equational mitoses to diploid nuclei that develop into the new macronuclei and micronuclei. Meanwhile, the macronucleus originally present in each conjugant disintegrates into many pieces which are eventually resorbed. In the process of exchange of gamete nuclei no cytoplasm (or effectively none) is normally exchanged. Conjugation thus provides for an exchange of genes, but no cytoplasm; and the resulting genotype of the two mates must be identical because each of the two haploid nuclei that unite to form the syncaryon in one conjugant has its exact copy in one of the two that unite in its mate. The cytological features of conjugation thus provide no basis for the observed difference in genetic phenomena among the varieties.

Nevertheless, as set forth above, exchange of genes alone brings about identity of characters in the two mates in variety 1 and not in variety 4. In the latter variety, identity of characters develops only in those exceptional matings in which both genes and cytoplasm are exchanged. It would seem as if the action of a gene in variety 1 must be equivalent to the action of a gene plus its cytoplasmic factor in variety 4. This relation is emphasized by the fact that entirely comparable characters, such as mating types or antigens, depend upon a gene only in variety 1 and upon a gene plus a cytoplasmic factor in variety 4. Further, in variety 4 different characters depend upon different cytoplasmic factors: each cytoplasmic factor is related to a particular gene. It seems improbable that a character, such as an antigen or a mating type, could in closely related varieties be the result of biochemical materials and processes so diverse that, in one variety, a gene alone is capable of controlling them while, in another variety, the gene requires in addition a cytoplasmic primer. These reflections forced me to consider the possibility that each gene in variety 1 includes what is distributed in variety 4 between a gene and a cytoplasmic primer. In other words, the cytoplasmic primers in variety 4 may correspond to a *part* of the gene in variety 1.

This possibility is obviously in conflict with what has been regarded as a fundamental conception in genetics: the indivisibility of the gene (see, e. g., Wright, '41). Is there any reason why separable parts of the gene might be discoverable in *Paramecium* and not in other kinds of organisms? The answer, it seems to me, is provided by consideration of the peculiar nuclear conditions characteristic of the ciliated Protozoa. Only in these organisms does there exist a distinction within each cell between a physiologically functional and a physiologically non-functional nucleus. The macronucleus is indispensable and controls the physiological activities of the cell; but the micronucleus is not essential: clones live and multiply well without it and maintain their genetic characters. Consequently, loss of physiologically important parts of the micronuclear genes would be of no importance to the cell. Hence the nuclear conditions in the ciliated Protozoa are such that loss of physiologically active parts of the micronuclear genes is a theoretical possibility. In other kinds of organisms such special nuclear

conditions do not exist and there is consequently no opportunity for gene disintegration to occur with impunity and so to be capable of detection.

By the same reasoning, however, one may conclude that the physiologically active macronucleus should in general retain the complete genes, for disintegration of these would be fatal to the organism. It would seem, therefore, that no essential difference should exist between the macronuclear genes in different varieties of *P. aurelia*. If the cytoplasmic primer in variety 4 corresponds to a part of the gene in variety 1, it should exist as a part of the macronuclear genes in both varieties.

This leads at once to the question of how the macronuclear genes could acquire their primer parts. There is no difficulty in variety 1 because both parts of the gene occur in the micronuclei which transform directly into macronuclei after fertilization. In variety 4, on the other hand, the micronuclei that give rise to macronuclei lack the primers. Previous experiments (Sonneborn, '43a) have shown, however, that the primers are in the cytoplasm at the time of fertilization. The new macronuclei at the time of their origin are therefore surrounded by cytoplasm containing the primers and could obtain them from this source. Further, the old macronucleus always disintegrates prior to the formation of new macronuclei, thus providing the cytoplasm with primers for the latter. The visible cytological processes accompanying fertilization thus supply a mechanism for the transfer of primers from macronucleus to macronucleus in those varieties in which the micronuclear source is cut off.

Here then is a clue to a mechanism of priming in variety 4. Gene K is the micronuclear gene which is normally transferred through the gamete nuclei at conjugation. In this form it is self-multiplying, both in micronucleus and macronucleus. If it unites, in the macronucleus, with kappa obtained from the cytoplasm, the complete gene is constituted—comparable to the micronuclear genes in variety 1—and undergoes self-duplication in the complete form. The necessity for priming arises from the separation of the parts of the gene and the inability of one part of the gene to produce the other part. In effect, K and K plus kappa are alleles and the change from one to the other is a mutation. The primary action of a gene, on this view, is self-duplication: K controls the production of kappa by reason of the fact that kappa becomes a part of the gene and thereby is reproduced as a part of it.

III. TESTS OF THE HYPOTHESIS

The preceding considerations seemed to justify the adoption, as a working hypothesis, of the assumption that K and kappa are capable of union and are in fact united in the macronucleus of killers. This hypothesis was subjected to the experimental tests presently to be set forth. To understand the tests, however, it is necessary first to recall certain important features of the macronucleus.

As set forth above, the macronucleus arises from the syncaryon as a simple diploid nucleus. It then grows enormously, becoming a multiple nucleus containing at least 30 units, each with a complete diploid set of genes. At each fission

the macronucleus divides amitotically, approximately half of the component unit nuclei passing to each daughter nucleus. However, the unit nuclei themselves must (on the basis of genetic evidence) divide by some sort of mitotic process, though the two products of division of a unit probably pass as a rule to the same daughter macronucleus. At times of fertilization, the compound macronucleus falls apart into its component units and these are resorbed in the cytoplasm.

With these features of the macronucleus in mind, the hypothesis that K and kappa are combined in the macronucleus could be tested if there were available a method of varying the amount of kappa introduced into non-killers containing gene K. For the K genes of the macronucleus may be considered as specific receptors for kappa and, if small enough amounts of kappa are presented to a macronucleus containing many K genes, there should not be enough to combine with all of these genes. Consequently, the amitotic divisions of the macronucleus during the course of repeated fissions would have to yield eventually some macronuclei completely devoid of kappa and some completely saturated with kappa. The former therefore could not yield killers, while the latter could.

The required method was developed by taking advantage of the following observation. When killers are crossed to non-killers, normally no cytoplasm (or effectively none) is exchanged and the conjugant pairs separate quickly after fertilization is completed: less than 3½ minutes elapse from the beginning of the separation process (at the anterior ends) until it is completed (in the region of the peroral cones across which the migratory gamete nuclei pass during fertilization). In the exceptional cases in which more than 30 minutes is involved from the beginning to the end of the separation process, cytoplasm is invariably exchanged and in amounts sufficient promptly to transform the non-killer mate into a killer. When the separation process takes an intermediate time, intermediate results are obtained presumably because intermediate amounts of kappa pass from the killer to the non-killer mate. This then provides a method of introducing reduced amounts of kappa into KK non-killers. However, the kappa is introduced at the time of fertilization and therefore before the syncaryon has produced the simple diploid nuclei from which the new macronuclei are to arise. Kappa is consequently present in the cell at the time the presumptive new macronuclei are in the simple diploid condition. Entrance of kappa into them at this time would result in macronuclei saturated with kappa. The results now to be set forth, however, indicate that the macronuclei may fail to become saturated. It appears, therefore, that kappa, when present in the cytoplasm in small amounts, does not necessarily get to the K genes of the new macronucleus before the latter begins to acquire its multiple condition. For the clones developed from such conjugants showed precisely the predicted segregation of kappa during the course of vegetative reproduction: lines of descent totally lacking kappa arose within these clones after from one to nearly 90 successive fissions, in different instances. Kappa, then, is unequally divided at fission in agreement with the amitotic division of the macronucleus.

While the preceding result was predicted on the basis of the hypothesis, the character of the lines which retained kappa (and the great majority did) was totally unexpected: they failed to become killers during long periods of asexual reproduction. That kappa was being produced and maintained all this time was nevertheless clearly demonstrated both by the ability of these non-killers to transmit kappa to other cells during conjugations involving cytoplasmic exchanges and by the fact that they usually yielded 100 per cent killer progeny when they underwent self-fertilization.

The 100 per cent maintenance of the non-killer character during vegetative reproduction and the 100 per cent transformation into killers after fertilization, while not foreseen and predicted, finds a simple explanation on the hypothesis under analysis. It is in fact precisely what would be required on this hypothesis, if the kappa combined with K in the macronucleus is unable to get back into the cytoplasm from the intact macronucleus, and if the killer character depends upon the presence of kappa in the cytoplasm. Under such conditions the non-killer character of the lines that maintain kappa is due simply to the combination of all the available kappa with K genes in the macronucleus leaving none (or effectively none) for the cytoplasm. The transformation of these non-killers into killers after fertilization would follow from the great excess of the kappa released into the cytoplasm at the time the compound macronucleus disintegrates, over the relatively small amount needed to saturate the K genes of the simple diploid or slightly compound macronuclei when they first develop after fertilization. As a consequence of the great disparity between the amount released by the many K genes of the old macronucleus and the amount with which the few K genes in the new macronuclei can combine, much is left over for the cytoplasm and the cell gives rise to a killer clone.

The observations on the consequences of introducing very small amounts of kappa into KK non-killer cells that previously lacked kappa are thus in agreement with the proposed hypothesis that K and kappa are united in the macronucleus when both are present in a cell. The observations have further indicated (1) that the kappa thus combined with K in the macronucleus does not escape from the intact macronucleus into the cytoplasm; and (2) that the phenotypic action of kappa depends on its being present in the cytoplasm. These ideas could be tested in another way if there were a method by which the number of K genes could be greatly increased without a corresponding increase in the amount of kappa. For this should lead by a different route to the same result as that obtained in the preceding experiments: in both cases the situation would be such as to yield some K genes lacking kappa.

This type of experiment is possible by taking advantage of the following known facts. At the time of fertilization, the old macronucleus, as has been said, breaks down into 30 or more pieces each of which contains at least one full diploid set of genes. Furthermore, the preceding experiments showed that KK non-killers containing kappa have kappa in the cytoplasm at this time. If, as the

experiments indicated, the kappa was previously combined with K in the macronucleus, the kappa must be released into the cytoplasm when the macronucleus disintegrates into pieces. During the course of the next two cell divisions, the new macronuclei which have arisen from the syncaryon presumably take up kappa. While the new macronuclei are growing and developing their normal compound condition, the pieces of the old macronucleus also grow though they are destined soon to be resorbed in the cell. This growth of the pieces of the old macronucleus involves at least a four-fold increase in volume during these first two cell divisions. This suggests that the genes of the macronuclear pieces undergo some multiplication after they have released kappa and before they are resorbed. At this stage, it is possible experimentally to suppress the division of the new macronuclei so that at the second postzygotic cell division some of the cells fail to get macronuclei. The pieces of the old macronucleus, however, are passively distributed (without division) among the daughter cells, so that each of the four cells gets about one-fourth of the pieces. In the cells that lack macronuclei but possess pieces of the old macronucleus, the latter not only fail to be resorbed, but each piece regenerates into a complete compound macronucleus (Sonneborn, '40, '42). The pieces are distributed at random during subsequent fissions until there is only one per cell, and thereafter this one, which by that time has reached full macronuclear size, divides normally at subsequent fissions. In this way, therefore, it is possible to get the many K genes of the pieces of the old macronucleus and the K genes they produced after they lost kappa to become functional and hence again to be receptors for kappa. In other words, many K genes normally destined to be lost are retained and multiplied and so provide a greatly increased number of kappa receptors.

This technique of increasing the number of receptor K genes was applied to animals of the pure killer race 51 in which non-killers had never before been found, in order to discover whether the increase in number of K receptors for kappa would yield any K genes lacking kappa. As in the previous experiments, macronuclear amitosis should eventually yield lines lacking kappa if any of the K genes of any of the regenerated macronuclei lacked kappa. The experiment did in fact yield both non-killers containing kappa (as in former experiments with introducing small amounts of kappa into KK non-killers) and non-killers from which kappa disappeared permanently, never reappearing in the course of subsequent vegetative or sexual reproduction. The experiments involving increase of K in killers thus leads to the same results as introducing small amounts of kappa into KK non-killers; both types of experiments yield the results predicted on the hypothesis that kappa is a dissociable part of gene K.

IV. DISCUSSION

A. *Darlington's views on the action of genes K and k.*—Darlington ('44) has suggested that kappa is not maintained by K, but is either inactivated by k

or is overgrown by a competitive plasmagene controlled by k. It has been shown above, however, that a pure killer race can be induced to yield a pure non-killer branch from which kappa permanently disappears without the introduction of k or any product of k into the organisms. This result renders Darlington's suggestion unnecessary and unlikely; and the means employed in the experiment to bring about the result support the alternative interpretation that K controls the increase of kappa. In a previous paper (Sonneborn, '43b) loss of killing action was reported in another race (47), and Darlington attributed this to hybridization with a race containing k. However, race 47 was isolated from all other races in the laboratory and had never been hybridized with another race during the years it was a killer or for a long time after it had become a non-killer. Nor has it at any time given any indication of containing gene k. In the absence of any evidence for Darlington's suggestion as to the role of k and, particularly, in view of the contrary evidence for the action of gene K, his interpretation seems unacceptable.

B. *Some unsolved problems.*—The major problem that remains unsolved is to account for the increase of cytoplasmic kappa. The material in sections II and III provides an explanation of how kappa primes K to produce more kappa in the macronucleus; but no data and no suggestions have yet been given as to how kappa is multiplied in the cytoplasm. It is known only that increase of kappa in the cytoplasm (which must occur in killer clones) depends upon the presence of K (and kappa?) in the macronucleus. The simplest suggestion appears to be that increase of cytoplasmic kappa may be brought about in essentially the same way as is the increase of macronuclear kappa, i. e., by combination with K, a part of K, or a product of K which, unlike kappa, is capable both of passage from the intact macronucleus to the cytoplasm and of very limited self-duplication in the cytoplasm. On this, however, there is as yet no experimental evidence.

Many other questions also need to be answered. Do the genes of variety 1 contain primers, as the hypothesis holds? Can alleles differ in the primer component only, i. e., can more than one kind of primer combine with the same basic part of the gene? If so, can two or more kinds of primers combine simultaneously with the same basic part? Is the primer alone the active part of the gene, or is the physiological specificity of the gene partly dependent on the basic gene also? These questions and others are now under investigation.

C. *Gene structure and action.*—The present paper presents and tests the hypothesis that the gene consists of two parts. One part, K in the example analyzed, is found in the chromosome, though its occurrence or the occurrence of part of it in the cytoplasm has not been excluded; the other part, kappa, occurs in both the chromosome and the cytoplasm. K can exist and multiply without kappa, but kappa cannot long exist or multiply without K. K alone produces no detected phenotypic effect; but kappa determines the killer phenotype. The possibility still remains that K not only controls the multiplication of kappa, but also, when in the presence of kappa, plays a part in the determination of the phenotype. The main features of gene action appear to be (a) self-duplication, and

(b) providing the cytoplasm with replicas of itself or part of itself. No effect of the gene on the phenotype is detected when the complete gene is confined to the macronucleus; only when at least kappa is also in the cytoplasm is phenotypic action discernible.

That the gene may consist of two diverse parts was suggested by Correns ('19) to account for variegation and by Thompson ('25, '31) to account for the Bar phenomena. In neither case was it possible to follow separately the assumed two parts of the gene, and the hypotheses were in the main formal and not subject to experimental test. That a primary action of the gene is the liberation into the cytoplasm of complete or partial copies has been suggested by Koltzoff ('35), Wright ('41) and others. This view, although based on weighty general considerations, has also not been subjected to experimental tests.

The work here reported on *Paramecium* supplies experimental tests indicating both the bipartite structure of the gene and the identity between an active cytoplasmic component and a part of the gene. The data, especially when they are presented in detail, need to be critically examined to see if there can be suggested some satisfactory alternative to the conclusion that K combines with kappa in the macronucleus. I have been unable to find one, but others may succeed where I have failed. In the absence of such an alternative, the present work may serve as a beginning of an experimental approach to the difficult problems of the structure and primary action of the gene.

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